Am. J. Trop. Med. Hyg., 86(4), 2012, pp. 561–565
doi:10.4269/ajtmh.2012.11-0504

Review: Controlled Human Blood Stage Malaria Infection: Current Status and Potential Applications

Christopher J. A. Duncan* and Simon J. Draper
Sir William Dunn School of Pathology, and Jenner Institute, University of Oxford, Oxford, United Kingdom

Abstract. Controlled human malaria infection by blood stage parasite (BSP) inoculation is an alternative to the well-established model of infection with *Plasmodium falciparum* sporozoites delivered by mosquito bites. The BSP model has been utilized less frequently, but its use is increasing. Advantages of BSP challenge include greater ease of administration, better standardization of the infecting dose per volunteer, and good inter-study reproducibility of *in vivo* parasite dynamics. Recently, a surprising reduction in clinical symptoms at microscopic patency in the BSP model has been identified, which has an undefined and intriguing pathophysiologic basis, but may make this approach more acceptable to volunteers. We summarize clinical, parasitologic, and immunologic data from all BSP challenges to date, explore differences between the BSP and sporozoite models, and propose future applications for BSP challenge.

INTRODUCTION

The role of controlled human malaria infection (CHMI) in testing efficacy of candidate vaccines and drugs against *Plasmodium falciparum* malaria is well-established.[1,2] A recent review has focused on the widely studied sporozoite (Spz) challenge model,[1] whereby persons are exposed to the bites of typically five malaria-infected mosquitoes.[3] An alternative model, involving inoculation of donor blood-stage parasites[4] (BSP), is used less frequently. Both methods were historically employed in malaria therapy for neurosyphilis with comparable clinical and parasitologic outcomes.[5]

CONTROLLED HUMAN BLOOD STAGE MALARIA INFECTION

The *P. falciparum* BSP model developed at the Queensland Institute for Medical Research in 1997 uses a cryopreserved stock of erythrocytes from a parasitemic donor.[4] Intravenous injection of a sterile suspension of thawed leukocyte-depleted erythrocytes, combined with monitoring of parasitemia post-inoculation by highly-sensitive quantitative polymerase chain reaction (qPCR), enables accurate calculation of *in vivo* parasite multiplication rates (PMRs), a surrogate end-point for vaccine efficacy testing.[4,6–9] The inoculum, estimated from the pre-freeze donor parasitemia, is retrospectively quantified by testing viability of the thawed stock.[4] Volunteers in BSP CHMIs were typically treated at a pre-defined parasite density predicted to prevent clinical malaria,[4,9,10] although more recent studies have used the onset of microscopic patency as a treatment endpoint.[6,11] The former approach generally avoids symptoms, with the ethical advantage of reducing volunteer discomfort, and the latter approach mirrors the more established Spz model and enables assessment of additional endpoints such as the microscopic pre-patent period.

Fifty-nine volunteers have been infected in this manner by using the same frozen starting material,[4,6,9–12] extending the safety database for this parasite stock. There is an extremely low risk of blood-borne virus transmission because of stringent screening of the original donor, and requirements for seropositivity for Epstein-Barr virus (EBV) and cytomegalovirus (CMV) in recipients (because of donor seropositivity).[4,11] Accumulated experience with BSP challenge over 14 years is summarized in Table 1. In studies that measure it, the microscopic pre-patent period is highly reproducible, suggesting overall maintenance of parasite growth and invasion characteristics despite prolonged storage. However post-thaw viability of the parasite stock varies widely (10–100%). This finding does not appear to be related to duration of storage, but may be caused by sensitivity of the parasites to freeze-thaw procedures and storage conditions,[9] and the current lack of an agreed or standardized viability assay.

Blood-stage vaccine assessment. Although BSP challenge bypasses the pre-erythrocytic stages of infection, it has a role in testing blood-stage vaccine efficacy.[6,9,11] The uniform inoculum simplifies PMR calculations and enables estimation of PMR with a greater degree of confidence (Figure 1A), and the low starting parasite load should enable detection of subtle effects on PMR with greater sensitivity than Spz challenge.[11] Contrasts between the models are summarized in Table 2. A major advantage is that BSPs (similar to cryopreserved Spzs) can be transported to areas without mosquito culture and infection facilities, and could therefore be used for vaccine efficacy testing in centers lacking a challenge suite. However, only two blood-stage (BS) vaccines have been tested by BSP challenge to date.[6,9] Although the small number of BSP challenge trials conducted reflects the relative paucity of Phase IIa CHMI trials of blood stage vaccines, there are several additional explanations (Table 2). These include negative impacts on volunteer acceptability from the perceived risks associated with receipt of a blood product, and the limitations on future blood donation imposed by blood transfusion services; the absence of regulatory approval in some regions (e.g., the United States); and the negative impact on volunteer eligibility arising from the requirement for recipient EBV and CMV seropositivity.[6]

In the two BS vaccine efficacy studies conducted by BSP challenge, vaccination did not reduce overall PMR[6,9] or delay time to microscopic patency.[6] A promising association between *in vitro* growth inhibition and *in vivo* PMR[6] needs to be replicated in other studies. However, in the absence of an efficacious BS vaccine capable of significantly reducing

*Address correspondence to Christopher J. A. Duncan, Sir William Dunn School of Pathology, University of Oxford, Oxford Molecular Pathology Institute Building, South Parks Road, Oxford OX1 3RE, United Kingdom. E-mails: chrisduncan@doctors.net.uk or christopher.duncan@path.ox.ac.uk

TABLE 1

Overview of published blood stage parasite controlled human malaria infection trials*

Reference	Cheng and others[4]	Lawrence and others[9]	Pombo and others[10]	Sanderson and others[11]	Duncan and others[6]	McCarthy and others[12]	All
Year	1997	2000	2002	2008	2011	2011	–
No. volunteers	5†	17	5	5	8	19	59
Primary objective	Pilot	Vaccine impact on PMR	Protection with repeated challenge	Pilot	Relationship of PMR to GIA	Kinetics of parasite clearance by antimalarial drugs	–
Center	QIMR	QIMR	QIMR	CCVTM	CCVTM	QIMR	–
No. parasites inoculated median (range)	3,000 (300–6,000)	127 (114–140)	30	1,800	250	Cohort 1: 360, Cohort 2/3: 1,800	360 (30–6,000)
Viability (%)	100	38	10	62	25	≈30	34 (10–100)
Treatment indication	3/5: MP, 2/5: 500–1,000 p/mL	PD (≈1,000 p/mL)	PD (≈1,000 p/mL)	4/5: MP, 1/5: Sympt.	8/8 - MP	PD 1,000 p/mL	43 PD, 15 MP, 1 Sympt.
Symptomatic at diagnosis	3/3‡	Treated at day 8 without symptoms	Treated at day 8 without symptoms	1/5	2/8	Cohort 1: Treated at day 6 without symptoms, Cohort 2/3: 4/13§	6/16 MP, 4/43 PD
Parasite density at treatment parasites/mL (median)	MP ≈10,000 PD 750	3,178 (peak)¶	913–3,000 (range)#	Not stated (> 10,000)	4,108	Cohort 1: 33, Cohort 2/3: 2,926 (peak)	MP ≈10,000 PD 1,964
Microscopic patency	3/3‡	NA	NA	4/5	8/8	NA	15/16
Pre-patent period (median)	8	NA	NA	8	Vaccine: 9, Control: 8.5	Cohort 1: NA, Cohort 2/3: (7 days to 1,000 p/mL)	8
PMR (median)	12.5	Not stated	NA	21.5	Vaccine: 17.5, Control: 17.6	Not stated	17.5
Vaccine	NA	Combination B	NA	NA	AMA1/C1+, CPG 7909	NA	–

*PMR = parasite multiplication rate per 48 hours; GIA = *in vitro* assay of growth inhibitory activity; QIMR = Queensland Institute for Medical Research, Brisbane, Queensland, Australia; CCVTM = Center for Clinical Vaccinology and Tropical Medicine, Oxford, United Kingdom; MP = microscopic patency; PD = parasite density threshold; p/mL = parasites/mL; Sympt. = treatment on symptoms and parasitemia (by quantitative polymerase chain reaction) before microscopic patency; NA = not available; AMA1 = apical membrane antigen 1.

†Two of five participants who had had malaria received their own autologous blood stage parasites, 1 of 5 malaria-naive participants received the blood stage parasite stock and was treated at blood film patency, and 2 of 5 malaria-naive participants were treated with 500–1,000 parasites/mL before symptoms or microscopic patency.

‡The denominator (3) includes only persons given a diagnosis by blood film microscopy (although 2 were not malaria naive and received their own autologous blood stage parasites).

§Dr J. McCarthy (unpublished data).

¶Day of diagnosis data not provided.

#Individual values and central tendency not provided.

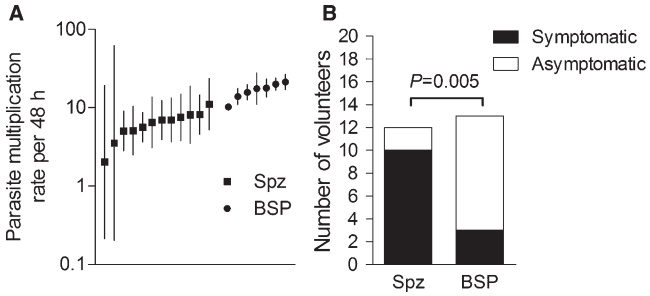

FIGURE 1. Confidence of parasite multiplication rate (PMR) calculations and frequency of malaria symptoms at blood film diagnosis. **A**, Individual PMRs ± 95% confidence intervals (modeled from quantitative polymerase chain reaction [qPCR] data as described)[6,7,11] for seven volunteers with sufficient data from a recent blood stage challenge trial[6] and unimmunized infectivity controls from a recently conducted sporozoite challenge study (n = 12 volunteers) (Ewer KJ, and others, unpublished data). See also Sanderson and others[11] for a similar analysis. **B**, Clinical symptoms from two blood-stage challenge trials conducted in Oxford (n = 13 volunteers)[6,11] and unimmunized infectivity controls from a recently conducted sporozoite challenge study (n = 12 volunteers) (Ewer KJ, and others, unpublished data). Two of eight[6] and one of five[11] volunteers were symptomatic in the respective blood stage parasite (BSP) infection studies compared with 10 of 12 sporozoite (Spz) infection volunteers. Methods of qPCR and clinical assessment of symptoms were identical in all studies. Significance testing by Fisher's exact test (Prism version 5.0).

PMR, possibly to a level approaching that estimated for semi-immune persons,[13] we cannot assess whether efficacy in the BSP model predicts efficacy against clinical malaria in the field. Without such a vaccine, the relationship between reductions in PMR, as measured by qPCR, and delay in time to a clinically-relevant outcome, such as microscopic patency[8,11] also remains experimentally unconfirmed. The absence of detection of a significant vaccine effect(s) in Phase IIa BSP challenge studies to date may also be seen as a limitation to the more widespread acceptance of this model in BS vaccine efficacy assessment, although this criticism could also be leveled at the Spz model. No blood stage vaccine candidate has yet provided convincing evidence of BS efficacy in Spz CHMI[14] although this is a more stringent model for BS vaccine efficacy assessment given the larger parasite load. Promising evidence for protective efficacy of vaccines targeting the leading blood-stage candidate antigen (apical membrane antigen 1)[15,16] (which is also involved in sporozoite invasion of hepatocytes)[17] suggests a potential added contribution from responses acting at the pre-erythrocytic stage(s), resulting in significant reductions in liver-to-blood inocula,[16] which would not be detected by BSP challenge. Ultimately, determination of the relative advantages of one model over the other in determining BS vaccine efficacy awaits the development of efficacious BS vaccines.

Pathophysiology. An interesting aspect of the BSP model is the apparent lower frequency of malaria symptoms compared with Spz challenge (P = 0.005) (Figure 1B), which appears unrelated to PMR[11] (Figure 1A), parasite density at diagnosis[6] or peak parasitemia (Figure 2). An intriguing possibility is that reduced diversity of *P. falciparum var* gene expression (observed after blood stage passage),[18] may lead to reduced switching of *P. falciparum* erythrocyte membrane protein 1, resulting in attenuated pathogenicity.[6] Conversely, the reduced diversity of *var* gene expression observed could reflect less host immune recognition. Antibodies against candidate vaccine

TABLE 2
Comparison of blood stage and sporozoite controlled human malaria infection models*

Advantages of BSP vs. Spz challenge			Disadvantages of BSP vs. Spz challenge		
Volunteer acceptability	Scientific quality	Practicalities	Volunteer acceptability	Scientific quality	Practicalities
No mosquito bites	Reduced inter-subject variability of parasite growth rate estimates	No requirement for gametocyte culture and mosquito infection facilities	Theoretical risk of pathogen transmission	Lack of pre-erythrocytic assessment	Requires sterile inocula preparation facilities, and liquid nitrogen for storage
Shorter duration of follow-up post-challenge	Highly reproducible pre-patent period		Requires IV cannulation	Attenuated pathogenicity?	FDA approval not granted for US trials
Efficient inoculation procedure	Blood stage–specific efficacy assessment		Future blood donation restricted in many countries	Induces immunity with repeated ultra-low dose?	EBV/CMV sero-restriction†
Reduced symptoms	Increased sensitivity to subtle blood-stage immune responses?				

*BSP = blood stage parasite; IV = intravenous; Spz = sporozoite; FDA = Food and Drug Administration; EBV = Epstein-Barr virus; CMV = cytomegalovirus.
†Specific only to current BSP stock.

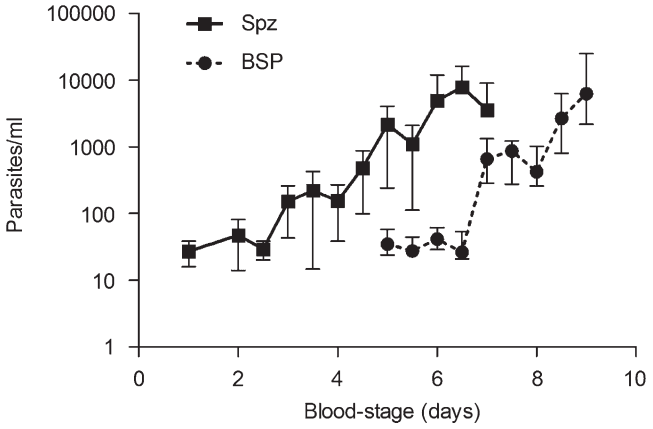

FIGURE 2. Comparison of *in vivo* parasite growth by quantitative PCR (qPCR). Mean ± 95% confidence intervals of parasitemia by qPCR in immunized and unimmunized volunteers infected with blood stage parasites (BSP) in Oxford (n = 8 volunteers)[6] were compared with unimmunized volunteers infected with sporozoites (Spz) from a recent study (n = 12 volunteers) (Ewer KJ and others, unpublished data) by identical qPCR assays. Day zero for BSP challenge corresponds to the day of inoculation, and equals day 6 post-Spz challenge when parasites may begin to seed the blood and monitoring of blood stage parasitaemia by qPCR begins. No significant difference in parasitemia at blood film diagnosis was observed. There is a prolonged sub-patent phase (undetectable by qPCR) of parasite growth after BSP inoculation (3–5 days), in comparison with the much shorter blood stage pre-patent period after Spz challenge (1–2 days).

antigen(s) were not increased in immunized persons after BSP exposure and were not detectable in unimmunized participants after the two reported Phase IIa challenge studies.[6,9]

Alternatively, the low starting parasite dose (median = 360 parasites) (Table 1), which results in a prolonged sub-patent phase of parasitemia undetectable by qPCR (3–5 days), could provide a greater window of opportunity than following Spz challenge for the acquisition of undefined anti-disease immunity (Figure 2) because in the Spz model, just one infected hepatocyte will seed the blood with ≈30,000 merozoites.[7] Similarly, there are indications that a larger BSP inoculum (3,000–6,000 parasites) may increase the frequency of symptoms.[4,12] Further work is thus needed to explore the phenotype of the host response to cryopreserved BSPs.

Blood stage CHMI has also provided some insights into malaria immunity. Protection against repeated low-dose BSP infection followed by drug cure was associated with parasite-specific T cell responses and nitric oxide synthase activity of mononuclear cells, but not antibodies.[10] However, prolonged *in vivo* effects of atovaquone are likely to have confounded the protection observed,[19] and to date this widely cited finding has never been replicated. Similarly, in a Spz challenge study, the BSP-specific T effector memory response was associated with protection in volunteers previously exposed to Spzs under chloroquine prophylaxis,[20] suggesting that a protective T cell response to BSPs may operate *in vivo*. An on-going BSP challenge trial will help to determine the stage-specificity of this protection (ClinicalTrials.Gov/NCT01236612).

FUTURE APPLICATIONS

One of the central unanswered questions in malaria immunology concerns the true nature of the protective immune responses *in vivo* in humans.[21] Spz and BSP CHMIs in semi-immune volunteers have significant potential to help address this question. A comparative approach using both models to infect semi-immune volunteers may dissect stage-specific determinants of natural immunity. Moreover, direct and accurate quantification of liver-to-blood inocula and PMRs in semi-immune volunteers would validate these models and set the goal posts for future prophylactic malaria vaccines. It should be noted that such studies would not be designed to provide efficacy assessment of candidate vaccines in target populations (e.g., non-immune infants), which would continue to be addressed by traditional Phase IIb studies.

To date, only three related parasite strains have been tested in CHMI.[1] A panel of diverse cryopreserved BSPs would enable assessment of cross-strain efficacy prior to large-scale Phase IIb field trials. New BSP stocks from EBV/CMV seronegative donors could also dramatically improve recruitment of volunteers in areas with low EBV/CMV seroprevalence.[6] Finally, a *P. vivax* Spz challenge model has also been recently developed[1] which requires a supply of infected donor blood for gametocyte propagation and mosquito infection. An alternative approach may involve directly preparing *P. vivax* BSP stocks from a carefully screened parasitemic donor.

CONCLUSIONS

Interesting and unanswered questions remain about the pathogenicity and immunogenicity of the BSP inoculum, and it is highly likely this model will continue to complement Spz challenge in assessing candidate BS vaccine efficacy, as well as providing insight into mechanisms of malaria immunity *in vivo* in humans.

Received August 3, 2011. Accepted for publication January 4, 2012.

Acknowledgments: We thank Professor Adrian V. S. Hill for helpful comments on the manuscript and Dr. James McCarthy for providing unpublished data.

Financial support: Christopher J. A. Duncan is supported by a Wellcome Trust Research Training Fellowship (094449/Z/10/Z) and Simon J. Draper is supported by a United Kingdom Medical Research Council Career Development Fellowship (G1000527) and is a Jenner Investigator.

Disclosure: None of the authors has any conflicts of interest.

Authors' addresses: Christopher J. A. Duncan, Sir William Dunn School of Pathology, University of Oxford, Oxford Molecular Pathology Institute Building, Oxford, United Kingdom, E-mails: chrisduncan@doctors.net.uk or christopher.duncan@path.ox.ac.uk. Simon J. Draper, The Jenner Institute, University of Oxford, Old Road Campus Research Building, Headington, Oxford, United Kingdom, E-mail: simon.draper@ndm.ox.ac.uk.

REFERENCES

1. Sauerwein RW, Roestenberg M, Moorthy VS, 2011. Experimental human challenge infections can accelerate clinical malaria vaccine development. *Nat Rev Immunol 11:* 57–64.
2. Moorthy VS, Diggs C, Ferro S, Good MF, Herrera S, Hill AV, Imoukhuede EB, Kumar S, Loucq C, Marsh K, Ockenhouse CF, Richie TL, Sauerwein RW, 2009. Report of a consultation on the optimization of clinical challenge trials for evaluation of candidate blood stage malaria vaccines, 18–19 March 2009, Bethesda, MD, USA. *Vaccine 27:* 5719–5725.
3. Herrington DA, Clyde DF, Murphy JR, Baqar S, Levine MM, do Rosario V, Hollingdale MR, 1988. A model for *Plasmodium falciparum* sporozoite challenge and very early therapy

of parasitaemia for efficacy studies of sporozoite vaccines. *Trop Geogr Med 40:* 124–127.

4. Cheng Q, Lawrence G, Reed C, Stowers A, Ranford-Cartwright L, Creasey A, Carter R, Saul A, 1997. Measurement of *Plasmodium falciparum* growth rates *in vivo*: a test of malaria vaccines. *Am J Trop Med Hyg 57:* 495–500.
5. Collins WE, Jeffery GM, 1999. A retrospective examination of sporozoite- and trophozoite-induced infections with *Plasmodium falciparum*: development of parasitologic and clinical immunity during primary infection. *Am J Trop Med Hyg 61(Suppl):* 4–19.
6. Duncan CJ, Sheehy SH, Ewer KJ, Douglas AD, Collins KA, Halstead FD, Elias SC, Lillie PJ, Rausch K, Aebig J, Miura K, Edwards NJ, Poulton ID, Hunt-Cooke A, Porter DW, Thompson FM, Rowland R, Draper SJ, Gilbert SC, Fay MP, Long CA, Zhu D, Wu Y, Martin LB, Anderson CF, Lawrie AM, Hill AV, Ellis RD, 2011. Impact on malaria parasite multiplication rates in infected volunteers of the protein-in-adjuvant vaccine AMA1-C1/Alhydrogel+CPG 7909. *PLoS ONE 6:* e22271.
7. Bejon P, Andrews L, Andersen RF, Dunachie S, Webster D, Walther M, Gilbert SC, Peto T, Hill AV, 2005. Calculation of liver-to-blood inocula, parasite growth rates, and preerythrocytic vaccine efficacy, from serial quantitative polymerase chain reaction studies of volunteers challenged with malaria sporozoites. *J Infect Dis 191:* 619–626.
8. Hermsen CC, de Vlas SJ, van Gemert GJ, Telgt DS, Verhage DF, Sauerwein RW, 2004. Testing vaccines in human experimental malaria: statistical analysis of parasitemia measured by a quantitative real-time polymerase chain reaction. *Am J Trop Med Hyg 71:* 196–201.
9. Lawrence G, Cheng QQ, Reed C, Taylor D, Stowers A, Cloonan N, Rzepczyk C, Smillie A, Anderson K, Pombo D, Allworth A, Eisen D, Anders R, Saul A, 2000. Effect of vaccination with 3 recombinant asexual-stage malaria antigens on initial growth rates of *Plasmodium falciparum* in non-immune volunteers. *Vaccine 18:* 1925–1931.
10. Pombo DJ, Lawrence G, Hirunpetcharat C, Rzepczyk C, Bryden M, Cloonan N, Anderson K, Mahakunkijcharoen Y, Martin LB, Wilson D, Elliott S, Eisen DP, Weinberg JB, Saul A, Good MF, 2002. Immunity to malaria after administration of ultra-low doses of red cells infected with *Plasmodium falciparum*. *Lancet 360:* 610–617.
11. Sanderson F, Andrews L, Douglas AD, Hunt-Cooke A, Bejon P, Hill AV, 2008. Blood-stage challenge for malaria vaccine efficacy trials: a pilot study with discussion of safety and potential value. *Am J Trop Med Hyg 78:* 878–883.
12. McCarthy JS, Sekuloski S, Griffin PM, Elliott S, Douglas N, Peatey C, Rockett R, O'Rourke P, Marquart L, Duparc S, Möhrle J, Trenholme KR, Humberstone AJ, 2011. A pilot randomised trial of induced blood-stage *Plasmodium falciparum* infections in healthy volunteers for testing efficacy of new antimalarial drugs. *PLoS ONE 6:* e21914.
13. Douglas AD, Andrews L, Draper SJ, Bojang K, Milligan P, Gilbert SC, Imoukhuede EB, Hill AV, 2011. Substantially reduced pre-patent parasite multiplication rates are associated with naturally acquired immunity to *Plasmodium falciparum*. *J Infect Dis 203:* 1337–1340.
14. Goodman AL, Draper SJ, 2010. Blood-stage malaria vaccines—recent progress and future challenges. *Ann Trop Med Parasitol 104*: 189–211.
15. Thompson FM, Porter DW, Okitsu SL, Westerfeld N, Vogel D, Todryk S, Poulton I, Correa S, Hutchings C, Berthoud T, Dunachie S, Andrews L, Williams JL, Sinden R, Gilbert SC, Pluschke G, Zurbriggen R, Hill AV, 2008. Evidence of blood stage efficacy with a virosomal malaria vaccine in a phase IIa clinical trial. *PLoS ONE 3:* e1493.
16. Spring MD, Cummings JF, Ockenhouse CF, Dutta S, Reidler R, Angov E, Bergmann-Leitner E, Stewart VA, Bittner S, Joumpan L, Kortepeter MG, Nielsen R, Kryzych U, Tierney E, Ware LA, Dowler M, Hermsen CC, Sauerwein RW, de Vlas SJ, Ofori-Anyinam O, Lanar DE, Williams JL, Kester KE, Tucker K, Shi M, Malkin E, Long C, Diggs CL, Soisson L, Dubois MC, Ballou WR, Cohen J, Heppner DG Jr., 2009. Phase 1/2a study of the malaria vaccine candidate apical membrane antigen-1 (AMA-1) administered in adjuvant system AS01B or AS02A. *PLoS ONE 4:* e5254.
17. Silvie O, Franetich JF, Charrin S, Mueller MS, Siau A, Bodescot M, Rubinstein E, Hannoun L, Charoenvit Y, Kocken CH, Thomas AW, Van Gemert GJ, Sauerwein RW, Blackman MJ, Anders RF, Pluschke G, Mazier D, 2004. A role for apical membrane antigen 1 during invasion of hepatocytes by *Plasmodium falciparum* sporozoites. *J Biol Chem 279:* 9490–9496.
18. Peters J, Fowler E, Gatton M, Chen N, Saul A, Cheng Q, 2002. High diversity and rapid changeover of expressed var genes during the acute phase of *Plasmodium falciparum* infections in human volunteers. *Proc Natl Acad Sci USA 99:* 10689–10694.
19. Edstein MD, Kotecka BM, Anderson KL, Pombo DJ, Kyle DE, Rieckmann KH, Good MF, 2005. Lengthy antimalarial activity of atovaquone in human plasma following atovaquone-proguanil administration. *Antimicrob Agents Chemother 49:* 4421–4422.
20. Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, van Gemert GJ, van de Vegte-Bolmer M, van Schaijk B, Teelen K, Arens T, Spaarman L, de Mast Q, Roeffen W, Snounou G, Rénia L, van der Ven A, Hermsen CC, Sauerwein R, 2009. Protection against a malaria challenge by sporozoite inoculation. *N Engl J Med 361:* 468–477.
21. Langhorne J, Ndungu FM, Sponaas AM, Marsh K, 2008. Immunity to malaria: more questions than answers. *Nat Immunol 9:* 725–732.